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Plant fibers for renewable growing media: Potential of defibration, acidification or inoculation with biocontrol fungi to reduce the N drawdown and plant pathogens

Bart Vandecasteele [a, *], Hilde Muylle [a], Imke De Windt [b], Joris Van Acker [b], Nele Ameloot [c], Kasper Moreaux [d], Peter Coucke [e], Jane Debode [a]

[a] Flanders Research Institute for Agricultural, Fisheries and Food Research (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 92, 9820 Merelbeke, Belgium
[b] UGent, Woodlab, Laboratory of Wood Technology, Department of Forest and Water Management, Coupure links 653, 9000 Gent, Belgium
[c] Greenyard Horticulture Belgium NV, Skaldenstraat 7a, 9042 Gent, Belgium
[d] Mycelia bvba, Veldeken 38A, 9850 Nevele, Belgium
[e] Wamcosa bvba, Boven Vrijlegem 41, 1730 Asse, Belgium

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ABSTRACT

Plant fibers allow for the partial replacement of peat in growing media and have potential to carry and sustain biocontrol organisms. However, they may also carry plant pathogens and they may vary in their degree of biodegradability and their interaction with N, and in this way thus affect the quality of growing media.

We tested 3 types of plant fibers (flax, miscanthus and reed) for replacement of peat in growing media. In order to (1) kill plant pathogens present on the plant fibers, (2) reduce the N fixation risk of the fibers and (3) provide fibers serving as carrier of biocontrol fungi, various defibration techniques were tested, including extrusion, retruding, disc refining and steam explosion.

Flax shives obtained from various sources were naturally colonized by viable microsclerotia of the plant pathogen *Verticillium dahliae*. When sufficiently high temperatures were reached during defibration, this technique killed the plant pathogen on the shives.

Untreated flax shives, miscanthus or reed straw were characterized by high N immobilization and thus reduce N availability for plants if used as growing medium. We found a clear positive correlation between the risk for N immobilization versus pH, the hemicellulose content and water-extractable C concentration of the fiber.

Unlike peat, defibrated pure miscanthus, reed straw and flax shives were easily colonized by fungal biocontrol strains. Inoculation of extruded miscanthus fibers with biocontrol fungi did not increase nor decrease the N immobilization. Reducing the pH of the fibers was effective in reducing the microbial activity and the N fixation.

Defibrated plant fibers have potential for peat replacement and as carrier for biocontrol fungi. Peat replacement is important in relation to the circular economy, climate change mitigation (prevention of carbon loss and greenhouse gas emissions) and the conservation of fragile ecosystems. Successful application of biocontrol fungi may reduce the need for using chemical crop protection agents in horticulture.
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1. Introduction

Although peat has many advantages for use in growing media (Bohlin and Holmberg, 2004), its extraction from pristine peatlands threatens these sensitive ecosystems and carbon sinks, meanwhile resulting in increased emissions of greenhouse gasses (Kern et al., 2017). Plant fibers such as miscanthus straw, reed and flax shives

Abbreviations: % N-immob., N immobilization; BAME, bacterial acid methyl ester; C_{-H2O}, waterextractable C; DM, dry matter; EC, electrical conductivity; FAME, fatty acid methyl ester; HC/L, holocellulose/lignin ratio; ME, extruded miscanthus straw; MEA, malt extract agar; ME-S, sterilized extruded miscanthus straw; MSEA, modified soil extract agar; NA, not assessed; OM, organic matter; PLFA, Total phospholipid fatty acid.

* Corresponding author. Burg. Van Gansberghelaan 109, 9820 Merelbeke, Belgium.
E-mail address: Bart.vandecasteele@ilvo.vlaanderen.be (B. Vandecasteele).

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allow for reducing or even completely replacing peat in growing media, but these materials may need optimization for water and air transport and water uptake capacity. These materials also vary in their interaction with N as N immobilization is of greatest concern for straw-based substrates (Altland and Locke, 2011). Results of Frangi et al. (2012), Guo-jing et al. (2001) and Debode et al. (2018) indicate a risk for N fixation when using miscanthus straw in growing media. Moreover, these straw-like fibers may have a lower biochemical recalcitrance and may thus have a faster decomposition rate, with a potential negative effect on the physical properties of the growing medium and plant development (Dannehl et al., 2015). N immobilization in growing media and composts has been related to microbial activity (Boyer et al., 2012; Buamscha et al., 2008; Jackson et al., 2009; Kristensen and McCarty, 1999; Vandecasteele et al., 2016). Results of Kaniszewski et al. (2010) indicate that not only the N availability but also the specific biochemical biodegradability of a material affects the microbial activity and thus its decomposition rate. The good positive correlation between C mineralization rate and biochemical composition (as indicated by the holocellulose/lignin ratio) had been reported previously during the composting process (Francou et al., 2008), and for silage, digestates and composts based on vegetable crop residues (Viaene et al., 2017). The effect of biochemical composition on the risk for N immobilization has been reported before for plant material (Jensen et al., 2005) or exogenous organic matter (Lashermes et al., 2010) incorporated in the soil.

Strategies to overcome N immobilization are based on adding additional N sources by adding more N by precharging the fibers with N fertilizers (e.g., with urea, Niemiera et al., 2014) or N containing acids (e.g., HNO_3; Kaniszewski et al., 2010), or focus on inhibiting microbial activity on the fiber, e.g., by installing a pH out of the optimal range (e.g., impregnation of wood material with sulfuric acid), or coating the fibers (Jackson et al., 2009; Kaniszewski et al., 2010).

Straw-like plant fibers such as flax shives have great potential as raw materials in a bio-based economy (Gao et al., 2017; Buranov and Mazza, 2008), but the fungal plant pathogen *Verticillium dahliae*, is becoming more and more prevalent among flax cultures in Northern Europe (Bressan et al., 2016). Since *V. dahliae* has a broad host range (incl. horticultural crops such as strawberry and ornamentals) and is found in the soil or within the flax shives as resistant microsclerotia, the prevalence of *V. dahliae* microsclerotia in flax shives limits the potential use of flax shives as raw material for growing media in horticulture. Also miscanthus and reed can be colonized by plant pathogens that may cause a threat for other crops (e.g., Covarelli et al., 2012; Crocker et al., 2015; respectively). Therefore, phytosanitary measurements are necessary in order to avoid the transmission of plant pathogens from plant fibers used as feedstock for growing media.

In order to condition the raw plant fibers for use in growing media blends different physical pretreatments can be envisaged. We define “defibration” as the transformation of straw-like materials into finer and smaller particles. Plant fibers and related biomass may be pretreated by steam explosion (Lizasoain et al., 2016; Menardo et al., 2013) or other defibration techniques, grinding or hammer-milling (Altland and Krause, 2009; Kuisma et al., 2014), or impregnation with N (Kaniszewski et al., 2010; Gruda et al., 2000). Defibration affects the behavior of the fibers in growing media by changing their chemical and biochemical properties, their disease suppressive capacity (De Corato et al., 2011), their susceptibility to microbial decomposition (Lizasoain et al., 2016; Menardo et al., 2013), their interaction with nutrients (Gruda et al., 2000) and risk for N immobilization (Gruda et al., 2000), their volume and density, their physical properties and particle size distribution. Lizasoain et al. (2016) and Menardo et al. (2013) reported that steam-exploded reed and miscanthus biomass had a higher digestibility during anaerobic digestion than untreated reed and miscanthus straw. Unlike the cellulose content, the hemicellulose and lignin content of reed straw was strongly affected by steam explosion, with up to more than 20% the hemicellulose fraction on dry matter (DM) basis being degraded into simple sugars (Menardo et al., 2013). Lizasoain et al. (2016) compared untreated and pretreated common reed biomass and concluded that steam explosion resulted in the substantial removal of hemicellulose, especially after pretreatments performed at 200 °C for 10 min or more.

Peat is not a good medium to harbor biocontrol fungi (application of beneficial fungi as a method for biological control of diseases and pests) as it does not provide the necessary compounds (carbohydrates, cellulose, chitin, lipids, etc.) for the fungi (Hoitink and Boehm, 1999; Krause et al., 2001). To optimize their application, carriers can be used to improve the survival and proliferation of biocontrol fungi in growing media. In the present study, we want to test if defibrated plant fibers serve as a good carriers for commercial fungal biocontrol strains. *Trichoderma* is the most widely known biocontrol agent in agriculture (Lorito and Woo, 2015) and several species have successfully been used against important fungal plant pathogens (Verma et al., 2007). Similarly, also species belonging to the genus *Chaetomium* and *Gliocladium* show antifungal activity against plant pathogenic fungi (e.g., Di Pietro et al., 1992; Papavizas, 1985). *Metarhizium anisopliae* is the most known *Metarhizium* species to be active against many insect pests (Zimmermann, 1993). The inoculation of plant fibers with these biocontrol fungi may affect the microbial activity on the plant fiber, and thus the risk for N immobilization: by adding more microbial biomass on the fibers by inoculation, this may potentially result in more microbial activity and the related effect on N immobilization.

The objective of the study is to assess the impact of physical, chemical and/or biological transformation of plant fibers on the suitability to replace peat in growing media. More specific, we assessed the impact of defibration, acidification or inoculation (as specific cases of physical, chemical and/or biological transformation) of reed straw, flax shives and miscanthus straw for use in growing media, with focus on the effect of transforming plant fibers on (a) the risk for N immobilization, (b) the risk for plant pathogens and (c) their potential as carrier for biocontrol fungi belonging to the genus *Trichoderma*, *Metarhizium, Chaetomium* and *Gliocladium*. The tested strategies to achieve these characteristics are:

- physical treatment: defibration using disc refining, retrusion, steam explosion, extrusion. These physical processes can induce high temperatures, thus affecting the risk for N immobilization and pathogens.
- chemical treatment: acidification of physical pretreated fibers with different acids to reduce the risk for N immobilization.

Shredded, hammermilled or extruded miscanthus straw was tested in growing media by several authors (Altland and Locke, 2011; De Corato et al., 2011; Frangi et al., 2012; Guo-jing et al., 2001; Kraska et al., 2018; Debode et al., 2018). But as far as we know, no experimental data are available on the use of reed straw or flax shives in growing media.

2. Methods

Several batches of flax shives, miscanthus and reed straw were chemically and biochemically characterized, and tested for the risk for N immobilization. These data were compared with compost, peat and wood fibers, i.e., other regularly used materials for

growing media blends (Fig. S1a).

The link between risk for N immobilization, pH, hemicellulose, cellulose and lignin content, biodegradation potential, C/N and water-extractable C for a dataset of 55 samples of raw materials (both peat and plant fibers) were tested by regression analysis to find out which characteristics indicate a higher risk for N immobilization (Fig. S1b).

The effect of four defibration techniques (disc refining, steam explosion, retruding, and extruding) on N immobilization and chemical properties were tested for the three plant fibers (Fig. S1c).

Five defibration techniques (disc refining, steam explosion, retruding, extruding, pelletisation) were tested for their effect on survival of microsclerotia of the fungal plant pathogen *Verticillium dahliae* on flax shives (Fig. S1d).

The inoculation success of defibrated plant fibers and peat with a fungal biocontrol strain (*Trichoderma harzianum* T22) was tested in vitro for three plant fibers and three defibration techniques (disc refining, steam explosion and extruding) (Fig. S1e).

The effect of acidification by two acids or inoculation of plant fibers with five biocontrol fungi on N immobilization was tested in incubation trials for extruded miscanthus straw. In these tests we also measured the microbial biomass on the plant fibers with two techniques (as N immobilization is related to microbial activity), and we assessed the reproducibility of the incubation trials (Fig. S1f).

2.1. Plant fibers and other raw materials for peat replacement in growing media

Plant fibers included in this study are miscanthus (*Miscanthus sinensis var. giganteus*) straw, reed (*Phragmites australis*) straw and flax (*Linum usitatissimum*) shives. Flax shives are a by-product of flax fiber extraction and consist of ligno-cellulosic xylem tissue from the inside of the flax stem. High amounts of flax shives are available in the flax-producer regions of France (100 000 tonnes/year), The Netherlands and Belgium (B, 35.000 tonnes/year). Several batches of flax shives from different locations in Belgium and the north of France were tested. The batches of reed straw were collected during the maintenance of water courses or nature conservation areas in Flanders (B), and the batches of miscanthus straw (including batches 1–4) were harvested in spring in a plantation at Wamcosa bvba in Mollem (B) and at ILVO in Melle (B). These batches were chopped during harvest in pieces of <4 cm.

Data on biochemical composition or the risk for N immobilization (see below) for several batches of unprocessed miscanthus straw, flax shives and reed straw were compared with data for (a) green waste composts (n = 27) from nine facilities collected at three periods during the year, (b) leaves and stalks (n = 20), and (c) cobs (without grains) (n = 20) for corn stover of four maize varieties collected at five locations, (d) several batches of wood fibers from different tree species (n = 20) and (e) several batches of peat from different origins (n = 10).

2.2. Risk for N immobilization and chemical characterization of plant fibers

Risk for N immobilization was assessed based on two different fertilizers, as fertilizer type may affect pH of the fibers. The fibers were tested for immobilization of mineral N by adding 350 mg N/L fibers as KNO_3 (Merck, pro analyse, >99%) or $(NH_4)_2SO_4$ (VWR International, pro analyse, >99.5%) followed by incubation at 37 °C for seven days (Fig. S2). By squeezing tightly a handful of the material (squeeze-ball test), it was determined if the material needed moistening before the incubation trial (when releasing at most only a few drops of water, the moisture content of the fibers is sufficiently high). The test was carried-out in triplicate; per replicate 200 ml material was used and 70 mg N was added by supplying 20 ml of a solution with 25.00 g KNO_3/L or 16.52 g $(NH_4)_2SO_4$/L. The material was put in a 250 ml polypropylene container covered with parafilm during incubation. Based on the difference between the theoretical (initial water-extractable mineral N concentration (EN 13652) + added amount of 350 mg N/L material) and actual water-extractable mineral N content after this incubation period, the % N immobilization was calculated. As such, 100% N immobilization means that all of the 350 mg mineral N/L material is immobilized. The protocol was successful in forecasting both the N immobilization in growing media during plant trials and the N immobilization in feedstock materials during compost trials (Vandecasteele et al., 2017a).

Together with the mineral N measurement at the beginning and the end of the incubation trial, electrical conductivity (EC) (EN 13038) and pH_{H2O} (EN 13037), and water-extractable C and P (EN 13652) was determined in a water extract (1:5 v/v). Water-extractable PO_4-P and NO_3-N was measured with a Dionex DX-3000 IC ion chromatograph (Dionex, Sunnyvale, CA). Water soluble NH_4-N was measured with a Skalar SAN++ flow analyzer (Skalar Analytical B.V, Breda, The Netherlands), and water-extractable C was quantified with ICP-OES. The water extracts were analyzed for their C content with or without filtration (<0.45 mm) on a membrane filter (MN Chromafil GF/P-45/25) to assess the size effect of the C extracted with water. Dry matter (DM) content, organic matter (OM) content and total N concentration (Thermo scientific – Dumas flash 4000 analyzer) of the plant fibers was determined according to EN 13040, EN 13039 and EN 13654-2, respectively. The C/N ratio was calculated by assuming that C = OM/1.80. Hemicellulose, cellulose and lignin content in the fibers was determined using an Ankom220 Fiber Analyzer extraction unit (Ankom, Macedon NY, USA) according to Van Soest et al. (1991). The holocellulose/lignin (HC/L) ratio as an indicator for the biodegradation potential (Francou et al., 2008; Viaene et al., 2017) of the fibers was calculated as (hemicellulose + cellulose)/lignin. Phytotoxicity of the defibrated plant fibers was tested with 3 replicates in a germination test with 20 seeds of *Lepidium sativum*.

2.3. Determination of microbial biomass – PLFA and fumigation-extraction

Total phospholipid fatty acids (PLFAs) were used to measure microbial biomass for extruded miscanthus fiber with or without inoculation with fungi or acidification, and for 7 different batches of peat. PLFAs were isolated from freeze-dried fibers using phosphate buffer, chloroform and methanol at a 0.9:1:2 ratio. Phospholipids separated by solid phase extraction were saponified to obtain free fatty acids, which were subsequently methylated using 0.2 M methanolic KOH to form fatty acid methyl esters (FAME), which were analysed with a capillary gas chromatograph-flam ionisation detector (Perkin Elmer Clarus 600, Perkin Elmer, Waltham, USA) with a col-elite-2560 column (100 m length x 0.25 mm ID, 0.25 μm film thickness, Perkin Elmer). PLFAs were identified and qualified from the retention time and response factor of each FAME in the external FAME and bacterial acid methyl ester (BAME) mix (Sigma Aldrich, St Louis, MO, USA).

Twenty PLFAs were selected because of their use of biomarker fatty acids for six distinct microbial groups: Gram-positive bacteria (i-C15:0, a-C15:0, i-C16:0, i-C17:0), Gram-negative bacteria (C16:1c9, C16:1t9, C17:0cy, C18:1c11, C19:0cy), bacteria (non-specific) (C14:0, C15:0, C16:0, C17:0, C18:0), actinomycetes (10Me-C16:0, 10Me-C17:0, 10Me-C18:0), fungi (C18:1c9, C18:2n9,12) and mycorrhiza (C16:1c11), and summed up to calculate total microbial biomass.

Microbial biomass C on plant fibers was measured by fumigating the soil mixtures with chloroform for 24 h in the dark and subsequent extraction with 0.5 M K_2SO_4 (1:2 fiber to extraction solvent ratio). The C contents of the fumigated and non-fumigated extracts were determined with ICP-OES. Microbial C was calculated as the difference in extractable C fraction of the fumigated and non-fumigated samples, including conversion factor 0.54 in the calculation of microbial C (Wu et al., 1990).

2.4. Physical treatment of fibers by defibration and plant pathogen detection

The defibration techniques for miscanthus straw, reed straw and flax shives included: extrusion with a PES 600-C-400 twin-screw Promeco Extruder System® at temperatures exceeding 100 °C, retrusion (Groencompost De Winter nv, Belgium), steam explosion in a 100 L Parr Reactor (Armstrong) (ULg, Belgium) and disc refining (Fraunhofer WKI, Germany). The effect of pelletisation (Flaxeco, Belgium) was only tested for flax shives (for plant pathogen detection). For assessing the number of viable *Verticillium* microsclerotia for flax shives from different origins and/or pretreatment, 1 g of each batch of flax shives was spread over 5 petri dishes containing modified soil extract agar (MSEA), a semi-selective medium for *V. dahliae* (Harris et al., 1993; Debode et al., 2011) and incubated at 22 °C. After 2 weeks, flax shives were carefully washed from the plates and clusters of viable *V. dahliae* microsclerotia were counted under a binocular. Chopped cereal straw was used as negative control as cereals are a non-host for *V. dahliae*.

2.5. Inoculation of plant fibers and peat with Trichoderma: in vitro test for inoculation success

We compared the potential of plant fibers based on miscanthus, flax shives or reed straw as carrier for biocontrol fungi with the carrier capacity of peat and vermiculite. For this test we selected the fungal biocontrol strain *Trichoderma harzianum* T22 (Trianum™, Koppert). In a preliminary test with several biocontrol fungi we observed a high colonization capacity for this fungus (Results not shown). Fibers were saturated with demineralized water for 60–90 min, and then water was allowed to drain for 120–150 min, and subsequently sterilized 48 h in closed glass bottles in an oven at 70 °C.

Two inoculation strategies were tested, i.e., malt extract agar (40/20) or sterilized grain as carrier for the inoculation. For the test with malt extract agar (MEA), *T. harzianum* T22 was inoculated on MEA in petri dishes, and after seven days the mycelium was used to inoculate the MEA of the test containers. After seven days of incubation 200 ml moistened and sterilized fibers were put on top of the biocontrol fungi. In this test inoculation success of miscanthus after disc refining was compared to peat and vermiculite. In a separate test, this methodology was used to compare inoculation by *T. harzianum* T22 on the 3 plant fibers (miscanthus, flax shives or reed straw) and 3 defibration techniques (steam explosion, disc refining and extrusion). Each test case counted 5 replicates.

For the test with *T. harzianum* T22 spawn on sterilized grain, 3 L wheat grains were boiled in rain water, and then 300 g wheat was mixed with 5 g poplar sawdust in each of the glass bottles, and bottles were sterilised in an autoclave for 20 min at 120 °C and at 1 bar. After cooling down, an 1 cm^2 piece of agar inoculated with *T. harzianum* T22 was put under sterile conditions on 1/3rd of the height of the grains in the bottle. The pots were shaken after *T. harzianum* T22 started to grow on the grains. After 7 days this spawn was used to inoculate the bottles with sterilized plant fibers. In this test inoculation success of miscanthus, flax shives or reed straw after disc refining was compared with peat and vermiculite. Per fiber type, four replicates were tested.

The growth rate of the biocontrol fungus was visually evaluated weekly for each culture vessel using the rating 1: low, 2: moderate or 3: high inoculation success. An average overall rate per week is given using equation (1):

$$Rate = \frac{\#\ high\ succes}{\#\ total} \times 3 + \frac{\#\ moderate\ succes}{\#\ total} \times 2 + \frac{\#\ low\ succes}{\#\ total} \times 1 \quad (1)$$

2.6. Effect of acidification or inoculation with biocontrol fungi on N immobilization in plant fibers

Acidification or inoculation may affect the microbial activity on the plant fiber, and thus the risk for N immobilization. Extruded miscanthus straw after inoculation with biocontrol fungi or acidification were tested for N immobilization in an incubation trial (see above). The tested biocontrol fungi were *T. harzianum* T22 (as above), *Metarhizium anisopliae* strain F52 (BIO1020®, Bayer Crop Science), *Chaetomium globosum* strain IHEM 3826 (BCCM), *Gliocladium catenulatum* strain J1446 (PRESTOP, Biobest), and *Trichoderma asperellum* T34 (Asperello™, Biobest).

The extruded miscanthus straw pre-inoculated with biocontrol fungi was produced in sterile conditions by Mycelia (Nevele, Belgium). Briefly, fungi were inoculated on (a) agar for *Metarhizium anisopliae*, *Chaetomium globosum*, *Gliocladium catenulatum* and *Trichoderma asperellum* or (b) spawn on sterilized grain starting from dedicated mother cultures for *T. harzianum*, and then were mixed with the sterilized (autoclaved) extruded miscanthus straw in a straw blender and incubated in Microsac gas-permeable bags (Saco2, Belgium) at room temperature for 11 days with the extruded plant fibers for further inoculation. The same technique was applied to produce extruded miscanthus straw intensively colonized with *Lentinula edodes* strain M 3782 based on spawn; this colonized fiber was only used for analysis of PLFA. Sterilized extruded miscanthus fibers were also acidified with an acetic or citric acid solution: distilled vinegar was mixed with the fibers, and 20 g citric acid pellets were dissolved in 100 ml tap water and then mixed with the fibers, respectively. This experiment was executed twice, for the second experiment the extruded miscanthus fiber was stored for one year and then sterilized before applying the acids.

2.7. Statistical analysis

Differences between miscanthus straw, flax shives, reed straw, green waste composts, wood fibers and peat for N immobilization (%) were tested using one way ANOVA, and Scheffé post-hoc tests (Statistica 13.1, Statsoft Inc.) after square root transformation and assuming homogeneity of variances and normally distributed populations. The link between risk for N immobilization, pH, hemicellulose, cellulose and lignin content, biodegradation potential (HC/L), C/N and water-extractable C for a dataset of 55 samples of raw materials were tested by correlation analysis (Pearson product-moment correlation coefficients (R) and the statistical significance of the correlation coefficient (p-value) were reported) and by stepwise forward linear regression (Statistica 13.1, Statsoft Inc.).

Differences between N immobilization (%) and pH after incubation for fibers inoculated with biocontrol fungi, and with or without acidification were tested per fertilizer type using one and two-way ANOVA, respectively, and Scheffé post-hoc tests (Statistica

13.1, Statsoft Inc.). Results for microbial biomass measured with PLFA and fumigation extraction were compared by correlation analysis (Pearson product-moment correlation coefficients (R)). Microbial, bacterial and fungal biomass (based on PLFA) before incubation, and after incubation with KNO_3 or $(NH_4)_2SO_4$ for 5 extruded miscanthus fibers, i.e., 4 inoculated fibers and the fiber without sterilization, were compared with one-way ANOVA and Scheffé post-hoc tests.

The variability and reproducibility of the incubation trial was assessed for the effect of extrusion and inoculation with *T. harzianum* T22, and for the effect of mineral N source, based on 3 separate incubation trials. Miscanthus straw with or without extrusion treatment and with or without inoculation with *T. harzianum* T22 and amended with 2 mineral N sources (KNO_3 versus $(NH_4)_2SO_4$) were tested in 3 separate incubation trials with three replicates each. As there was an interaction effect of the applied fertilizer, the data were tested with two-way ANOVA for the effect of treatment and trial for each fertilizer type separately. N immobilization was tested with two-way ANOVA and Scheffé post-hoc tests for the factors 'Trial' and 'Fiber', without significant effect of the trial on the N immobilization ($p > 0.05$).

3. Results

3.1. Biochemical composition and risk for N immobilization

Clear biochemical differences between the 3 plant fibers in this study and other regularly used materials for growing media (peat, green waste compost, wood fiber) or other plant fibers (i.e., corn stover) were observed (Fig. 1). Mainly reed and miscanthus straw clearly have a higher biodegradation potential (as indicated by the higher HC/L ratio) than green waste compost, or pure green waste, peat and wood fibers, as was assessed by comparison of characteristics of different batches (Fig. 1). The 3 plant fibers in this study clearly show higher risk for N immobilization than peat and wood fibers (Fig. 1): the risk for N immobilization was significantly higher for flax shives and miscanthus straw than for peat and wood fiber ($p < 0.01$) For reed straw the risk for N immobilization was only significantly higher than for peat ($p < 0.05$). Green waste composts have a high range of values for N immobilization due to different compost characteristics; the risk for N immobilization was significantly positive related to microbial activity in these composts (Vandecasteele et al., 2016).

Data on risk for N immobilization, pH, hemicellulose, cellulose and lignin content, biodegradation potential (HC/L ratio), C/N ratio and water-extractable C were available for a dataset of 55 samples of raw materials (reed and miscanthus straw, flax shives, peat and wood fibers). A high positive correlation was observed between the risk for N immobilization on one hand, and pH-H_2O (R: 0.68, $p < 0.05$), hemicellulose (R: 0.63, $p < 0.05$) and water-extractable C (R: 0.66, $p < 0.05$), on the other. By stepwise forward linear regression the best model for prediction of the risk for N immobilization ($R^2 = 0.65$) was found for a model based on these characteristics, and with % lignin and C/N ratio (equation (2)):

$$\%\,\text{Nimmob} = -51.2 + 0.43 \times \text{pH-H}_2\text{O} + 0.38 \times \text{C-H}_2\text{0} + 0.29 \times \%\,\text{hemicellulose} + 0.11 \times \%\text{lignin} - 0.11 \times \text{HC/L} - 0.19 \times \text{C/N}\ (n = 55,\ p < 0.001) \quad (2)$$

With the p-value for pH-H_2O, C-$H_2$0, %hemicellulose, %lignin, HC/L and C/N being 0.004, 0.001, 0.023, 0.28, 0.38 and 0.07, respectively.

We conclude that reed, miscanthus straw and flax shives clearly show higher risk for N immobilization than peat and wood fibers,

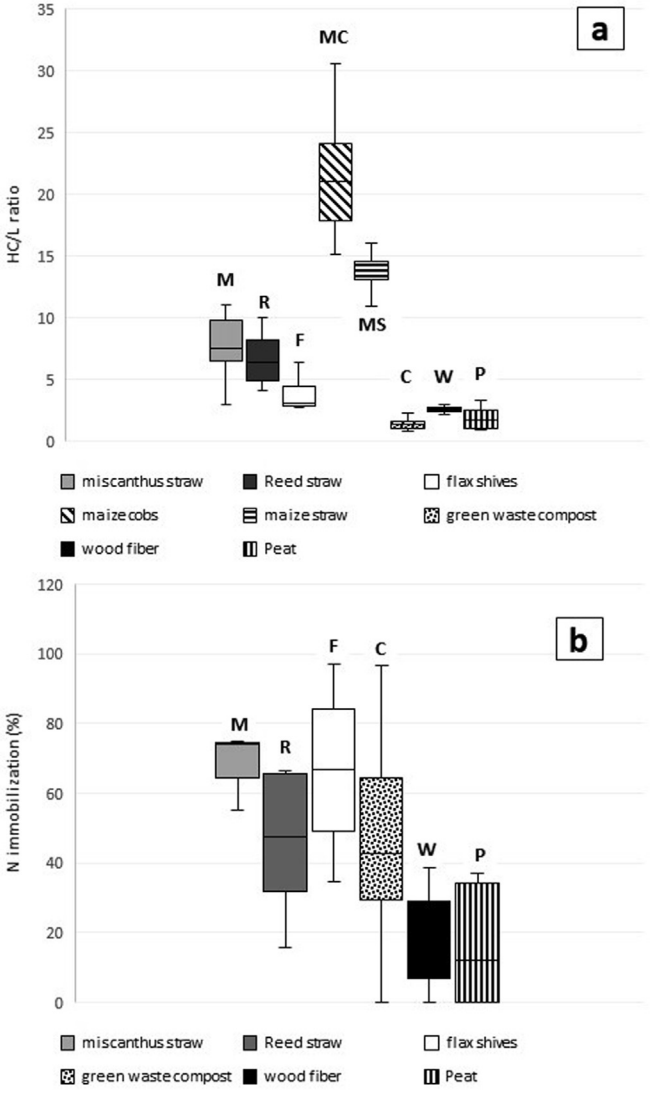

Fig. 1. Boxplots for different batches of biomass for (a) holocellulose/lignin ratio (HC/L) for unprocessed miscanthus (M) straw ($n = 19$), flax (F) shives ($n = 9$), reed (R) straw ($n = 9$), green waste composts (C) ($n = 27$), maize leaves and stalks (ML, $n = 20$), and cobs (MC, without grains) ($n = 20$), batches of wood (W) fibers ($n = 20$) and peat (P, $n = 10$), and (b) % N immobilization tested with KNO_3 for unprocessed miscanthus straw ($n = 5$), flax shives ($n = 7$), reed straw ($n = 8$), green waste composts ($n = 27$), wood fibers ($n = 20$) and peat ($n = 10$). Negative values for N immobilization indicate net N mineralization.

and that pH-H_2O, hemicellulose and water-extractable C were found to be the best predicting characteristics for clarifying this risk. The water extracts were also analyzed for their C content after filtration (<0.45 mm) to determine the total dissolved C concentrations in the extract, with no significant effect ($p > 0.05$) on the amount of C measured. These results show that the C compounds extracted with water are very small in size, i.e. the extracted C is mainly total dissolved C, highly available to bacteria and fungi.

3.2. Effect of defibration on N immobilization and plant pathogens

We found specific effects of type of fiber and defibration technique on N immobilization (Table 1). In some cases, defibration also changed the main characteristics of the fibers, such as DM content, water-extractable C or pH (Table 1), and indirectly affecting the microbial activity on the fiber and the risk for N immobilization,

Table 1
Chemical and biochemical composition of different plant fibers before and after processing with defibration techniques (DM: dry matter, HC/L: holocellulose/lignin ratio, OM: organic matter, N immob.: % N immobilization tested with KNO_3, EC: electrical conductivity, NA: not assessed, C-H_2O: waterextractable C). N immobilization was tested in 3 replicates per fiber. Different batches of flax shives, miscanthus and reed straw were indicated by different numbers ([a]: tested for *Verticillium dahliae* microsclerotia, [b]: tested for inoculation success on malt extract agar, [c]: tested for inoculation success for *T. harzianum* spawn on sterilized grain, [d]: germination success of *Lepidium sativum*).

Fiber	Treatment	% N-immob.	Hemicellulose	Cellulose	Lignin	HC/L	OM	DM	C/N	pH-H_2O	EC	C-H_2O
			%/DM	%/DM	%/DM	–	%/DM	%/fresh		–	µS/cm	mg/l substrate
Miscanthus1	Untreated [d]	74.3	27.5	54.8	10.9	7.6	98.1	86.2	191.9	6.9	87	913
	Extrusion [d]	43.4	27.0	50.1	12.2	6.3	97.7	53.3	171.2	7.3	116	751
	Disc refining [b,c,d]	38.1	30.3	51.9	11.3	7.2	96.8	76.6	190.0	7.3	116	343
Miscanthus2	Untreated	55.0	29.9	51.1	11.3	7.2	97.2	86.9	188.2	6.7	250	868
	Steam Explosion[d]	75.4	14.0	51.9	10.6	6.2	97.2	24.8	213.4	4.0	381	3564
Miscanthus3	Untreated	73.7	20.0	46.4	10.6	2.9	96.6	75.5	119.5	7.9	164	383
	Retruder	45.0	25.0	50.0	13.6	5.8	96.3	80.5	98.3	8.1	264	604
	Disc refining	38.7	24.0	52.1	14.6	5.5	97.5	88.9	109.0	6.8	98	404
Miscanthus4	Untreated	74.9	25.0	49.7	11.6	5.6	96.8	81.7	101.7	7.2	186	455
	Disc refining	33.3	24.0	51.1	12.6	5.4	96.6	89.0	109.7	6.7	112	469
Reed1	Untreated [d]	65.7	27.9	38.8	11.8	5.7	87.0	52.1	58.9	6.9	118	365
	Extrusion [d]	69.3	28.5	28.9	23.5	2.4	88.9	58.4	63.7	6.7	165	642
	Disc refining [c,d]	66.5	28.0	38.4	12.0	5.5	87.4	73.6	54.1	7.0	218	965
	Steam Explosion [d]	93.3	7.9	41.6	12.1	4.1	91.7	30.0	65.1	3.7	300	2639
Reed2	Untreated	42.8	29.0	47.7	18.6	8.2	95.1	83.7	93.2	6.0	58	255
	Retruder	86.5	26.5	35.9	19.6	5.9	74.8	67.9	68.3	6.7	229	1023
	Disc refining	21.5	29.0	52.5	20.6	7.8	96.8	88.2	111.1	5.6	49	308
Flax shives1	Untreated [a,d]	59.8	21.0	58.1	12.5	6.3	97.0	87.7	76.5	7.2	105	903
	Extrusion [a,d]	65.5	22.0	55.0	13.2	5.8	94.9	73.5	78.1	8.0	136	966
	Disc refining [a,c,d]	85.4	23.5	44.5	23.5	2.9	96.4	77.2	76.4	7.4	139	882
	Steam Explosion [a,d]	31.7	10.6	41.2	24.8	2.1	96.1	27.3	132.5	4.5	383	5214
Flax shives2	Untreated [a]	34.8	25.0	51.3	15.6	5.6	96.4	58.5	90.5	7.3	45	355
	Retruder [a]	67.4	21.0	44.4	17.6	3.0	93.8	63.5	107.2	7.1	110	770
	Disc refining [a]	20.5	20.0	54.4	16.6	4.2	98.4	88.3	133.0	6.3	53	434

thus changing its potential for use in growing media. After steam explosion, the risk for N immobilization increased for miscanthus and reed straw, but not for the flax shives. Steam explosion resulted in a strong decrease in hemicellulose content, a strong increase in water-extractable C, and a clear decrease of the pH, and a clear reduction of plant germination, pointing at phytotoxicity (Table S1). The N immobilization of miscanthus straw is significantly reduced when using extruding, retruding or applying disc refining without affecting the pH of the fiber, while steam explosion significantly reduced the pH, and resulted in higher N immobilization. For the reed straw and flax shives, the effects of the defibration on N immobilization were not as straightforward: for batch Reed2 the N immobilization increased after the retruder process but decreased for disc refining. For batch flax shives1 N immobilization increased for disc refining and decreased for steam explosion, while a different behavior was found for the batch flax shives2. The variable effects of the combinations of batches and processes indicate the process-specific conditions to interact between the same raw material and process.

Next to effects on N immobilization, the hygienisation due to the defibration was tested. All 8 batches of untreated flax shives (Table 2) were colonized by viable *V. dahliae* microsclerotia from 7 to 168 microsclerotia g^{-1}. Three of the four tested defibration techniques were able to kill the microsclerotia, as no viable *Verticillium* colonies were counted for these treatments (0 microsclerotia g^{-1}), while for the retruded flax shives the number of viable *Verticillium* colonies was still high (89 microsclerotia g^{-1}). A similar hygienisation effect was achieved when pelleted flax shives were tested. No viable microsclerotia were detected on the untreated cereal straw (negative control).

Table 2
Number of viable microsclerotia of the fungal plant pathogen *Verticillium dahliae* on flax shives after 2 weeks of incubation at 22 °C on petri dishes containing Modified Soil Extract Agar (each value is the result of 5 petri dishes).

Fiber	Treatment	Verticillium
		# viable microsclerotia/g fiber
Flax shives1	Untreated	10
	Extrusion	0
	Disc refining	0
	Steam Explosion	0
Flax shives2	Untreated	101
	Retruder	89
	Disc refining	2
Flax shives3	Untreated	168
	pelletized	0
Flax shives4	Untreated	135
	Pelletized	1
Flax shives5	Untreated	7
Flax shives6	Untreated	7
Flax shives7	Untreated	40
Flax shives8	Untreated	8
Cereal straw	Untreated	0

3.3. Inoculation of plant fibers with the fungal biocontrol strain *T. harzianum T22*

Both for the inoculation test with *T. harzianum* T22 on malt extract agar (Fig. 2A) or the inoculation test with *T. harzianum* T22 as spawn on sterilized grain (Fig. 2B) it was observed that inoculation of *T. harzianum* T22 was faster for plant fibers after disc refining than for peat or vermiculite, indicating that these plant fibers are a better carrier for biocontrol fungi than peat or vermiculite. Inoculation was slower for vermiculite than for the plant fibers in both trials, and was decreasing after 1 or 2 weeks for the first and second trial, respectively (Fig. 2). The inoculation of peat was initially better in the first trial, but in both trials decreased after 1–2 weeks. In another test with *T. harzianum*, we found no significant differences in inoculation success between flax shives, reed straw or miscanthus processed by steam explosion, disc refining or extrusion, with scores of 2.6 or higher (Table S3).

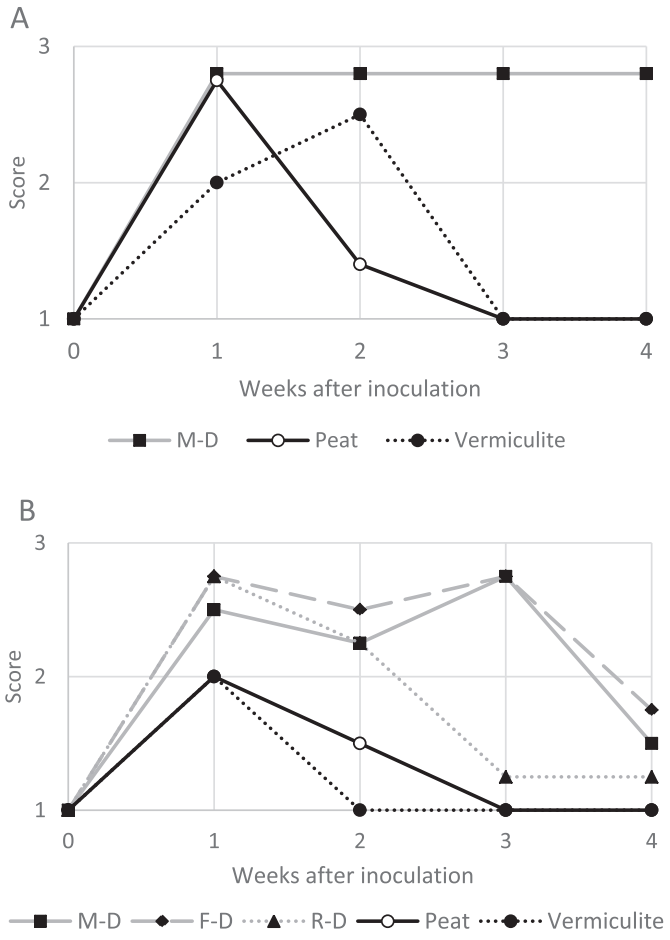

Fig. 2. Evolution of the scoring of inoculation success of A) miscanthus (M) after disc refining (D), peat and vermiculite inoculated with *T. harzianum* on malt extract agar or B) miscanthus, flax shives (F) or reed straw (R) after disc refining, peat and vermiculite inoculated with *T. harzianum* spawn on sterilized grain. Scores are based on (A) 5 (malt extract agar) or (B) 4 (spawn) replicates per treatment (1: low, 2: moderate or 3: good inoculation success).

3.4. Incubation trial: robustness and effect of temperature and fertilizer

Further tests were based on extruded miscanthus straw, as this combination of plant fiber and defibration technique resulted in lower risk for N immobilization (Table 1). To assess the variability and reproducibility of the incubation trial, miscanthus straw with or without extrusion treatment and with or without inoculation with *T. harzianum* T22 and amended with 2 mineral N sources (KNO_3 versus $(NH_4)_2SO_4$) were tested in 3 separate incubation trials with three replicates each.

Both for KNO_3 and $(NH_4)_2SO_4$ there was no significant effect of the trial on the N immobilization ($p > 0.05$). From these results we conclude that the incubation trial is robust for predicting N immobilization, even in case of fungal inoculation. In case of KNO_3, N immobilization was significantly higher for the miscanthus straw (70%) than for the extruded miscanthus straw without inoculation (40%) or with inoculation of *T. harzianum* T22 (54%), based on the Sheffé post-hoc comparison ($p < 0.01$). In case of $(NH_4)_2SO_4$, N immobilization was significantly higher for the miscanthus straw (77%) than for the extruded miscanthus straw without inoculation (50%), which also was significantly higher than for the miscanthus with inoculated with *T. harzianum* (40%), based on the Sheffé post-hoc comparison ($p < 0.01$).

3.5. Effect of inoculation with biocontrol fungi on N immobilization

Inoculation of extruded miscanthus fibers with different strains of biocontrol fungi did not increase nor decrease the N immobilization: none of the biocontrol fungi resulted in a significantly higher or lower risk for N immobilization in comparison with the sterilized extruded miscanthus straw, for both of the fertilizers (Table 3).

PLFA was used to measure the microbial biomass on the extruded miscanthus fiber with or without inoculation with fungi, i.e., to assess changes in total microbial, fungal or bacterial biomass and, in case of inoculation with biocontrol fungi, to have an indication of the inoculation success. For 9 of these fibers with different pretreatment or inoculation before incubation, total microbial biomass was also measured with the fumigation-extraction method for validation. We observed a significant positive correlation ($p < 0.05$, $R = 0.84$, $n = 9$) between the total microbial biomass C as measured with the fumigation-extraction method (range between 100 and 1145 μg C/g) and the total microbial biomass as measured by PLFA (range between 673 and 2676 nmol/g DM). Both methods are thus able to quantify the differences in total microbial biomass between plant fibers.

The pure miscanthus straw before defibration had a low total microbial biomass as measured with PLFA (177–777 nmol/g DM, Table S1). In general, total microbial biomass as measured with PLFA was higher for the inoculated fibers (1060–10930 nmol/g DM) (Tables 3 and 4 and Table S2). Two exceptions were extruded miscanthus straw stored for one year and not being sterilized (fiber without inoculation, Table S2), and extruded miscanthus straw inoculated with *M. anisopliae* (Table 3). The first material had been colonized by a green fungus (probably *Penicillium sp.*) during long-term storage and already contained 1240 nmol/g DM of microbial biomass at the onset of the incubation trial. Extruded miscanthus straw was only very slowly inoculated with *M. anisopliae*, resulting in a low concentration of microbial biomass (764 nmol/g DM) at the

Table 3
Initial microbial biomass and pH and N immobilization (% N-immob.) and pH after incubation of extruded miscanthus straw (ME), and sterilized extruded miscanthus straw (ME-S) with or without inoculation with biocontrol fungi. N immobilization was tested with 2 sources of mineral N as fertilizer, and significance of the effect of treatment was tested per fertilizer type (N source) with one-way ANOVA. Values per treatment for N immobilization (%) and pH after incubation are averages of 3 replicates, while initial microbial biomass and pH were measured for one replicate. Different letters indicate significant differences according to the Scheffé post-hoc test (DM: dry matter).

Treatment	Microbial biomass (nmol/g DM)	pH-H2O init.	pH-H_2O	% N-immob.	pH-H_2O		% N-immob.
			KNO_3		$(NH_4)_2SO_4$		
ME	1240	6.8	8.1	37	5.4	b	58
ME-S	674	5.3	7.6	57	4.4	a	59
ME-S-*Trichoderma harzianum*	2766	6.7	7.9	36	6.9	c	39
ME-S-*Metarhizium anisopliae*	764	6.6	7.6	42	5.7	b	44
ME-S-*Trichoderma asperellum*	1098	6.2	8.6	75	6.1	bc	39
ME-S-*Chaetomium globosum*	1660	6.7	8.7	70	6.2	c	44
ME-S-*Gliocladium catenulatum*	1060	6.7	7.6	53	6.1	bc	38

Table 4
Initial microbial biomass and pH, and N immobilization (% N-immob.) and pH after incubation of sterilized extruded miscanthus straw (ME-S) with or without acidification by acetic or citric acid addition, tested in 2 incubation trials (Trial I and II) with 2 sources of mineral N as fertilizer. Significance of the effect of treatment and fertilizer (N source) were on N immobilization (%) and pH after incubation were tested with two-way ANOVA. Values per treatment for N immobilization (%) and pH after incubation are averages of 3 replicates, while initial microbial biomass and pH were measured for one replicate. Different letters indicate significant differences according to the Scheffé post-hoc test (DM: dry matter, NA: not assessed).

Treatment	Trial	Microbial biomass (nmol/g DM)	initial pH-H2O	pH-H_2O				%N-immob.		
				KNO_3		$(NH_4)_2SO_4$		KNO_3	$(NH_4)_2SO_4$	
ME-S	I	674	5.4	8.6	d	5.7	F	63	56	d
ME-S + citric acid	I	184	2.6	2.5	a	2.5	A	14	14	a
ME-S + acetic acid	I	684	4.3	4.7	c	4.3	D	18	26	ab
ME-S	II	777	5.3	7.6	d	5.4	E	58	58	d
ME-S + citric acid	II	788	4.1	4.1	bc	4.1	C	31	36	bc
ME-S + acetic acid	II	673	2.9	3.1	ab	2.9	B	37	38	c
Treatment				**				**		
Fertilizer				NS				NS		
Fertilizer × treatment interaction				**				NS		

start of the incubation trial (for this fiber also a low value for microbial C was measured with the F-E method). PLFA data indicated high microbial biomass on fibers with *L. edodes* (i.e., intensively colonized extruded miscanthus straw, Table S2), and for the fiber with *T. harzianum* (Table 3).

For 5 fibers, i.e., 4 inoculated extruded miscanthus fibers and the extruded miscanthus fiber without sterilization, PLFA were measured before incubation, and after incubation with KNO_3 or $(NH_4)_2SO_4$ (Table S2). The fungal biomass was significantly ($p < 0.001$) lower after incubation with KNO_3 (125 nmol/g DM) or $(NH_4)_2SO_4$ (106 nmol/g DM) than for the fibers before incubation (902 nmol/g DM). The total bacterial and total microbial biomass were not significantly different after incubation with KNO_3 (665 and 790 nmol/g DM) or $(NH_4)_2SO_4$ (782 and 888 nmol/g DM) than for the fibers before incubation (665 and 1547 nmol/g DM), respectively. These results indicate a decline in fungal biomass during incubation, while total bacterial and total microbial biomass were not significantly affected by incubation.

In the incubation test for N immobilization, two fertilizers were used: KNO_3 and $(NH_4)_2SO_4$. For the fibers with initial pH > 5, both fertilizers significantly ($p < 0.001$) affected the pH during the incubation trial. The average pH increase during incubation was 1.3 pH units in case of KNO_3 while the average pH decrease during incubation was 1.1 pH units in case of $(NH_4)_2SO_4$. In contrast, for the fibers with pH < 5, i.e., the fibers already having a low pH, both fertilizers did not significantly ($p > 0.05$) affected the pH during the incubation trial (Table 3).

Changes in mineral N forms may indicate changes in oxygen availability during the incubation trial. For the treatments fertilized with KNO_3 less than 7% of the mineral N was measured as NH_4-N at the end of the incubation trial, for the treatments fertilized with $(NH_4)_2SO_4$ less than 5% was available as NO_3-N, except for some treatments: higher NH_4-N concentrations were only measured for the fibers with *T. harzianum*. These results indicate that there was only substantial oxygen consumption by microbial respiration for the fibers inoculated with *T. harzianum* resulting in transformation of NO_3-N into NH_4-N, which is indicative for partly anaerobic conditions during the incubation trial for these treatments. For the other treatments, the mineral N was not transformed in the treatments with low or no N immobilization, i.e., the treatments with still high mineral N concentrations at the end of the incubation trial. For NO_3-N this illustrates that no anaerobic conditions took place during the incubation trial.

3.6. Effect of acidification of plant fibers on N immobilization

For the tested defibration techniques, only steam explosion leads to a strong pH decrease of the fibers (Table 1), but also to a strong increase of the N immobilization, thus this defibration technique is not effective in decreasing the risk for N immobilization. Here we test the effect of other acidifying treatments of the fibers, i.e., using acetic and citric acid, on N immobilization. In comparison with sterilized extruded miscanthus straw, the reference treatment, clearly lower N immobilization was observed for the acidified fibers, and their initially low pH did not change during the incubation trial. Application of KNO_3 clearly increased the pH for the sterilized extruded miscanthus treatment without added acids, but no effect of KNO_3 application for the other treatments and no effect of $(NH_4)_2SO_4$ was observed (Table 4). Reducing the pH of the fibers to values between pH 2.5 and 4.7 by acidification with acetic or citric acid was thus effective in reducing the N drawdown.

PLFA was used to measure the microbial biomass on the extruded miscanthus fiber with or without acidification, i.e., to assess changes in total microbial, fungal or bacterial biomass. Microbial biomass based on PLFA in the first batch with citric acid was low, while the microbial biomass in the second batch with citric acid and acetic acid was clearly higher (Table 4). This might be related to the ME being stored after the first trial for one year before sterilization for the second trial.

For the treatments fertilized with KNO_3 in Table 4 less than 7% of the mineral N was available as NH_4-N at the end of the incubation trial; for the treatments fertilized with $(NH_4)_2SO_4$ less than 5% was available as NO_3-N. These results indicate that the mineral N was not transformed, even in the treatments with low or no N immobilization. For the KNO_3 treatments this indicates that no anaerobic conditions took place during the incubation trial as NO_3-N was not transformed to NH_4-N. For the $(NH_4)_2SO_4$ treatments we conclude that no nitrification activity took place, which is probably related to the high temperatures, restricting the nitrification activity.

4. Discussion

When assessing the quality and potential of materials for peat replacement, we need to balance several technical aspects, e.g.:

- No risk for plant pathogens and potential use as carrier for biocontrol fungi
- Interaction with nutrients and reduction of the risk for N immobilization

4.1. No risk for plant pathogens and carrier for biocontrol fungi

Direct heat treatment of biowaste can be used to destroy plant

pathogens. This study shows that all defibration techniques, except retruding, were effective in inactivating the *V. dahliae* microsclerotia on the flax shives. The failure of the retruding technique is most likely related to the lower process temperature during retruding versus the other techniques, i.e., appr. 50 °C for retrusion versus >100 °C for the other defibration techniques. *Verticillium* microsclerotia are melanized resting structures and thus very recalcitrant against environmental stress (Bell and Wheeler, 1986; Debode et al., 2007). If microsclerotia are killed by defibration, then less robust plant pathogen structures (e.g. conidia, mycelia) are certainly inactivated. Defibration of plant fibers at sufficiently high temperatures (100 °C) is thus sufficient to avoid problems with plant pathogens in general and this is in accordance to the requirements of the European and Mediterranean Plant Protection Organization for the treatment of biowaste of plant origin to ensure its phytosanitary safety use in horticulture (OEPP/EPPO, 2008). The recommended time/temperature combination is 70 °C for 1 h, preferably by wet heat. However, for the heat treatment to be effective, the particle size of the biowaste should preferably not be larger than 12 mm. The biowaste should accordingly be homogenized. During the heat treatment, the moisture content of the biowaste should be sufficient to guarantee heat transfer between and inside the particles. The specified temperature should apply to the whole bulk of material over a continuous period of time.

Inoculation of plant fibers with biocontrol fungi increases the disease suppressiveness of growing media blends with these plant fibers. This was demonstrated by Debode et al. (2018) who incorporated 20% *Trichoderma* pre-colonized extruded miscanthus straw in peat. Post-harvest disease incidence of the strawberry fruits was decreased and defense-related genes in the leaves were induced in the *Trichoderma* pre-colonized miscanthus straw treatment as compared to the miscanthus straw without *Trichoderma* pre-colonization or when *Trichoderma* spores were added directly to the peat-based growing medium. Accordingly, in the present study, we found that the plant fibers with higher HC/L ratios are more easily colonized by biocontrol fungi as compared to the conventionally used organic or mineral materials for growing media such as peat and vermiculite (Fig. 2). Fibers with higher HC/L ratios may be prone to a higher risk for N immobilization. Total microbial biomass on the extruded miscanthus fibers was measured with PLFA and was significantly positive correlated with biomass as assessed with the fumigation-extraction method. We measured microbial biomass also on 7 batches of different types of peat, with values in the range of 154–468 nmol/g (Table S2). PLFA measurements were useful to assess microbial biomass on the fibers, and only in the case of sterilized or acidified extruded miscanthus fibers low PLFA concentrations were measured. Inoculation resulted in higher microbial biomass, depending on the inoculation success (Table 3 and Table S2). The measured PLFA concentrations in the inoculated plant fibers were clearly higher than measured concentrations in peat for the total fungal, total bacterial and total microbial biomass (Table S2), indicating that bacteria and fungi are abundant on the fibers. N immobilization is the result of microbial decomposition, thus the higher initial microbial biomass on fibers due to fungal inoculation may affect this process, i.e., it may potentially further increase the risk for N immobilization. We observed that inoculation of defibrated plant fibers with biocontrol fungi did not increase nor decrease the N immobilization, illustrating that these fibers are good carriers for biocontrol fungi without increasing the risk for N immobilization although these fibers host a high microbial biomass. The inoculation with biocontrol fungi does not increase the decomposition rate of the fibers, as illustrated by the results of the N immobilization test.

4.2. N immobilization

An issue with the use of renewable growing media blends is the potential retention of nutrients in the organic substrates, as well as their degradability, which may affect the physicochemical properties of the growing medium (Domeno et al., 2009). For a greenhouse with 400 m^3 growing medium per ha, 100% N immobilization as assessed in the protocol used in this paper points at a N fixation of 140 kg N/ha. Kuisma et al. (2014) reported that hammer-milled straw of reed canary grass with a C/N ratio of 23 could successfully be used in growing media, without problems of N immobilization. The extruded miscanthus straw used in our trial had a C/N ratio of 190, resulting in higher risk for N immobilization in the growing medium. In a previous experiment (Vandecasteele et al., 2017b) we noticed that hammer-milled miscanthus straw had a clearly lower risk for N immobilization, i.e., 32%, than for the batches of untreated miscanthus straw in this study (Fig. 1).

The risk for N immobilization by pure plant fibers does only indicate the potential risk for N immobilization by fiber-amended growing media, with the actual effect being depending on the volume% fiber in the mixture and the N supply during cultivation. Both factors will also determine how reversible the lower pH in acidified plant fibers and the related N immobilization can be maintained. However, acidification did not always result in a reduced risk for N immobilization, as was illustrated by the steam-exploded miscanthus straw: although steam explosion resulted in a pH decrease, this decrease seemed not sufficient to reduce the N immobilization. Extrusion and disc refining of the miscanthus straw did not affect the pH of the fibers.

The effect of shredded, hammermilled and extruded miscanthus straw in growing media on plant yield was tested by Kraska et al. (2018), Altland and Locke (2011), Frangi et al. (2012) and Debode et al. (2018). No effects of N immobilization in blends with 20–80% miscanthus straw were reported by Altland and Locke (2011), but the slow release fertilizer was intentionally placed close to the roots of the young plants to reduce the effect of N immobilization. Kraska et al. (2018) did not report negative effects on tomato yield for drip-fertigated miscanthus shreds, chips or fibers versus rockwool growbags. In contrast, plant growth was significantly reduced due to lower N content in growing media with 25–75% extruded miscanthus and *Arundo donax* L. fiber compared with the peat-based substrate (Frangi et al., 2012). Debode et al. (2018) showed that N immobilization by 20% extruded miscanthus straw reduced strawberry growth and yield in 1 out of 2 experiments. Increasing the dose of N fertilization (Jackson et al., 2009; Sharman and Whitehouse, 1993) or improving the accessibility of the fertilizer for the plant roosts (Altland and Locke, 2011) may alleviate this risk of N immobilization.

4.3. pH of plant fibers

The pH of the growing media affects several processes, including nutrient availability and uptake by plants, and microbial activity. The protocol used in this study includes a pH measurement before and after the incubation trial. The applied source of mineral N in the test affected the pH of the material, and may thus also affect the microbial activity and thus the related N immobilization. The results suggest that the effect of the applied mineral N fertilizer should be taken into account for extrapolating the results of the protocol to substrate cultivation: the expected effect may be affected by the pH changes induced by the fertilizer amendment to the growing medium.

The buffering capacity of the tested fibers seems to be low, as strong pH changes were observed due to inoculation, fertilizer application or acidification. On the other hand, the pH obtained by

acidification with acetic acid or citric acid was maintained during incubation, without significant effect of the added fertilizer. Previous research indicated that composts may have a high buffer capacity for acidification (Costello and Sullivan, 2014), affecting their potential as carrier for biocontrol fungi or for peat replacement.

Other techniques for acidification are elemental S addition and silage. Ensiling may be an appropriate technique for storage, resulting in a temporary pH decrease of the silage, but silage additives were found to be necessary to effectively ensile miscanthus straw (Whittaker et al., 2016). Silage might be a cheaper processing technique than acidification, but we did not observe a pH decrease nor a decrease in risk for N immobilization when the extruded miscanthus straw was ensiled (results not shown), indicating that more research is needed on the need for silage additives (Whittaker et al., 2016). Future research should also focus on changes in pH during crop cultivation, i.e., on the reversibility of the pH of plant fibers in growing media blends. During cultivation, plants may also affect the pH of the growing medium in a species or even cultivar-specific way (Dickson and Fisher, 2017).

4.4. Microbial decomposition and biochemical composition

N immobilization occurs when organic materials with high C/N ratios, such as straw and other plant fibers, are microbially degraded (Jackson et al., 2009; Vandecasteele et al., 2016), leading to competition with crops for the available N, and is thus triggered by microbial activity. It is important to assess potential limitations for microbial decomposition of the plant fibers and the resulting reduced risk for N immobilization. Potential limiting factors are: macronutrient shortage (N, P, K), limited C availability, pH beyond optimal range for microbial activity, O_2 or water shortage, absence of microbial biomass (Domeno et al., 2009) or inhibition due to chemical components.

Stability and degradability of growing media should be assessed after N addition, to overcome N shortage, limiting the decomposition activity (Domeno et al., 2011). As N was added in readily available forms and in sufficient concentrations, it should not be a limiting element. P concentrations were not found to be a limiting factor for microbial activity during the incubation trial, as the measured water-extractable P concentrations at the end of the incubation trial in case of fungal inoculation or acidification were clearly higher than the limit of detection, i.e., >5 mg P/l substrate (results not shown). Based on the water-extractable N and P concentrations at the end of the incubation trial in the fibers with a low N immobilization (<40%), an average N/P ratio of 6 was measured. This is in line with the N/P ratio of 4.9 for microbial biomass (Griffiths et al., 2012) and may indicate optimal P concentrations and an optimal N/P ratio for microbial growth. In the case of using KNO_3, K was added as well in an easily available form, thus K availability is not considered to be a limiting factor for microbial activity neither. Only for the acidified fibers, pH was beyond the optimal range for microbial activity. In the protocol a water check is included and, if needed, the fibers are hydrated at the onset of incubation. O_2 shortage was not observed during the incubation trials, as NO_3-N was not transformed into NH_4-N during the incubation trials. Unlike pH, the effect of type of N fertilizer had only a limited effect on the results of the incubation trials for N immobilization, indicating that the results were robust. Buamscha et al. (2008) tested also 2 N forms, i.e., KNO_3 and NH_4NO_3, with no effect of N form at the tested concentration of 75 mg/l on the measured N immobilization and microbial activity. The data for water-extractable C indicate that C concentrations were sufficiently high to avoid that C availability was a limiting factor.

The biochemical composition also affects the microbial decomposition activity (Francou et al., 2008; Viaene et al., 2017). Changes in biochemical composition during defibration may affect the potential for microbial decomposition. Steam explosion resulted in a strong decrease in hemicellulose content for the three fibers (Table 1), as reported before by Lizasoain et al. (2016) and Menardo et al. (2013) for reed and miscanthus biomass, respectively. These changes are affected by process temperature: according to Garrote et al. (1999), hemicellulose begins to solubilize at 150 °C. Second, the breakdown of long hemicellulose chains into simple sugars increased the easily degradable substrate for the microorganisms, and we measured very high concentrations of water-extractable C for the steam-exploded fibers (Table 1). Nevertheless, steam explosion can result in degradation products such as furan derivatives, which may have an inhibitory effect on the microorganisms e.g., involved in anaerobic digestion (Lizasoain et al., 2016, Menardo et al. (2013) or on plant germination (Table S1). De Corato et al. (2011) concluded that steam-exploded miscanthus straw could be used against some soil-borne fungal diseases in growing media, and its suppressiveness could be related to the concentration of the microbial inhibitors produced during steam explosion. However, the high N immobilization (Table 1) and phytotoxicity (Table S1) observed in our study may indicate potential negative effects for plants growing on the growing media with these fibers, and N immobilization may be related with the observed disease suppression.

Future research should focus on: (a) the effect of hammermilling versus defibration techniques on N immobilization and inoculation success, (b) buffer capacity of plant fibers for pH changes, and the reversibility of the pH of plant fibers in growing media blends during crop cultivation, (c) testing defibrated plant fibers with and without biocontrol fungi in greenhouse trials with different crops for effects on plant growth, and crop quality and health, and (d) testing shelf life of growing media blends with defibrated plant fibers with and without biocontrol fungi for chemical and physical properties.

5. Conclusions

Extruding, retruding and applying disc refining significantly reduced the risk for N immobilization for miscanthus straw without affecting the pH of the fiber, while steam explosion significantly reduced the pH and resulted in higher N immobilization. Effects for reed straw were less straightforward, while extruding and retruding the flax shives increased the N fixation.

The extrusion of miscanthus straw with or without inoculation of a biocontrol organism reduced the N immobilization when compared with the pure miscanthus fiber. In case of flax shives, defibration of plant fibers kills plant pathogens that may be present on the fibers when sufficiently high temperatures are reached during defibration.

Inoculation of plant fibers with biocontrol fungi may be beneficial for increasing the disease suppressiveness of growing media. The effect of acidification or colonization by biocontrol fungi as strategy to reduce the risk for N immobilization was tested for extruded miscanthus straw. Acidification of extruded miscanthus fibers reduced the N immobilization, whereas colonization by biocontrol fungi did not increase nor decrease the N immobilization. The use of defibrated and inoculated plant fibers in growing media will reduce the demand for peat and close resource loops, which will benefit the environment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2018.08.167.

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